

USE OF FRESHWATER MUSSELS (BIVALVIA: UNIONIDAE)
TO MONITOR THE NEARSHORE ENVIRONMENT OF LAKESRoger H. Green,* Robert C. Bailey,* Scott G. Hinch*†,
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ABSTRACT. A combination of observational studies and manipulative field, mesocosm, and laboratory experiments have shown that lentic populations of unionid mussels, in particular *Lampsilis radiata* and *Elliptio complanata*, respond to environmental variation in several ways. Thus, mussels may be useful as monitors of their environment. Shell morphology, degree of shell etching, and shell growth rates vary along a gradient of exposure to water energy. These phenotypic responses to environmental variation appear to have little genetic basis. Two polymorphic allozyme loci were examined with electrophoresis, and allelic frequencies showed little spatial pattern. The heritability of shell size and shape was assessed and found to be quite low. However, in transplant experiments mussels moved to different environments were strongly influenced by the environment from which they came. For example, growth rate and tissue metal burdens at the end of a 1-year transplant study are determined much more by the source lake than by destination lake. This "source effect" can be explained by (a) slowly reversing acclimation of a common genotype to contrasting habitats (e.g., north shore Lake Erie and adjacent waters), or (b) underlying but as yet undetected genetic differences which are a product of selection in genetically isolated populations (e.g., separate lakes on an acidity gradient in the Muskoka/Haliburton region). Our attempts to use contaminant levels in the mussel shell as an environmental monitor were not successful. However, the research does demonstrate that changes in density and growth rate parameters may be attributable to pollution, despite the potentially confounding effects of natural environmental variation.

ADDITIONAL INDEX WORDS: Benthos, benthic environment, indicators, contaminants.

INTRODUCTION

This paper presents the results of research on the use of unionid mussels as response systems in environmental monitoring studies. Bivalve molluscs have long been used for pollution monitoring in the marine environment, and recent work has become quite sophisticated (e.g. Bayne *et al.* 1985, 1988).

Some arguments for the use of bivalve molluscs in pollution studies include (Green *et al.* 1985):

(1) they typically inhabit and feed from the shallow nearshore environments which are both most productive and most threatened by pollution.

(2) they are relatively sedentary,

(3) they are relatively long lived,

(4) their shells can provide a physical and per-

haps chemical record of past ambient environments in which they have lived,

(5) they are well studied taxonomically, physiologically, and (relative to most marine or freshwater taxa) genetically, and

(6) they are known to take up or otherwise be affected by contaminants.

The potential for the use of bivalve molluscs in freshwater environments has not been overlooked (e.g., Smith *et al.* 1975, Anderson 1977, Heit *et al.* 1980, Leard *et al.* 1980, Imlay 1982, Koide *et al.* 1982). However, freshwater studies have been less sophisticated than marine work—especially regarding carefully formulated hypotheses, good sampling and experimental design, and planned experiments in both field and laboratory to complement observational data. In freshwater environments emphasis has been mostly on collecting bivalves from polluted environments and assessing some aspect of the community or a particular population (e.g., species composition, tissue contaminant levels, size distributions). Usually these

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results are compared to those from "clean" sites. Little has been done to carefully separate effects of pollution from other potentially confounding factors such as physiology, genetics, age, or natural environmental variation. Transplants from clean to polluted sites have often been done in a way that tacitly assumes mussels are "sponges," without complex physiological processes which are in part genetically based.

An ultimate goal is to build models which predict the responses of mussels from the properties of the natural environment and permit tests of hypotheses related to the additional effect of pollution-associated factors. The general objective of this paper was to initially assess the use of unionid mussels as "response systems" for use in freshwater environmental studies. This required combining results from several specific studies which:

- (1) assessed the phenotypic plasticity of unionid mussels when subjected to varying environmental conditions.
- (2) assessed whether responses to natural environmental variation can be distinguished from "pollution" effects.
- (3) validated the aging of mussels from shell growth rings so that age-specific responses to contaminants could be detected,
- (4) determined whether or not the chemical composition of the shell can be related to pollution that occurred during the life of the mussel,
- (5) quantified the genotypic component of the response of mussels to the environment (with emphasis on larval development and heritability of morphological ontogeny), and
- (6) evaluated the influence of known pollution situations on growth rate, shell morphology, shell chemistry, and soft tissue chemistry.

PHENOTYPIC PLASTICITY IN RESPONSE TO ENVIRONMENT

The phenotypic plasticity of freshwater mussels in response to varying natural environmental conditions has been inferred by many malacologists who have described intra-specific variability of shell form as a function of various environmental factors. Some of our own earlier research dealt with the description of variability in shell form of a species as a function of varying chemical and physical environment (Green 1972, Bailey *et al.* 1983). Our recent research has focused on two unionid species, *Lampsilis radiata* (studied mostly along

the Lake Erie north shore) and *Elliptio complanata* (studied mostly in lakes of the Muskoka, Haliburton, and Algonquin Park regions). Both are abundant and widespread species, eminently suitable for monitoring for environmental quality. The response of both *Lampsilis* spp. and *Elliptio* spp. to environmental variation has often been studied (Green 1971, 1972; Smith *et al.* 1975; Anderson 1977; Green 1980; Heit *et al.* 1980; Leard *et al.* 1980; Tessier *et al.* 1984; Dermott and Lum 1986; Czarnezki 1987; Servos *et al.* 1987).

Studies by Bailey (1987, 1988) showed that the unionid community of Inner Long Point Bay, Lake Erie, is diverse (10 species collected). Within the inner bay there is a gradient of exposure to wind and waves, and analysis of morphological variation in the shell of one species, *Lampsilis radiata*, showed a gradient of biological response coinciding with the "exposure gradient." *L. radiata* had heavier shells and faster growth rates at the high exposure end of the gradient (Bailey and Green 1988). A reciprocal transplant experiment (Hinch *et al.* 1986), involving large numbers of marked *L. radiata* either moved or left as controls for most of the open water season, showed that mussels moved to the more exposed site grew differently in shape. Overall growth rate was related to the source (low vs. high exposure sites) of the transplanted mussels.

Hinch (1987) studied *E. complanata* in lakes of the Muskoka/Haliburton region threatened in varying degrees by acidification. Before assessing effects of acidification on the mussels, Hinch conducted several studies to evaluate effects of natural environment variation, especially exposure to water turbulence, that might be confounded with effects of water chemistry change driven by acidification. In 1985, Hinch sampled 30 lakes which had been surveyed by the Ontario Ministry of Environment (A.P.I.O.S. 1981-83). Four of these lakes (Green, Beech, Bark, and Tock) were selected for intensive study, based on similarity of lake morphology, ease of access, presence of *E. complanata* populations, and coverage of a wide range of alkalinity and pH (pH: 7.5, 7.2, 6.3, and 6.0; alkalinity: 243, 238, 153, and 22 $\mu\text{eq/L}$, respectively). Mussels were collected from a site on the lee shore and a site on the windward shore of these lakes, and shells were compared among lakes (representing water chemistry effects), between sites within lakes (representing effects of exposure to water turbulence), and among mussels within sites (Hinch and Bailey 1988). "Exposure sites" within

TABLE 1. Mean area of *Elliptio complanata* shell that is etched in three lakes at sites differing in exposure to water turbulence. At each site $n = 25$ mussels.

Lake	pH	Exposure	Mean area etched (mm ²)	Standard error
Tock	6.0	high	801.77	57.95
Tock	6.0	low	300.33	29.33
Bark	6.3	high	475.84	45.55
Bark	6.3	low	368.86	26.05
Beech	7.2	high	1,216.83	98.43
Beech	7.2	low	429.08	76.65
Green	7.5	high	638.03	77.47
Green	7.5	low	277.74	34.95

lakes differed in sediment type, as one would expect protected and exposed shallow water sites. Far more of the variation in shell form (including size variation) was due to between-site exposure differences (ca. 34%) than to among-lake chemical differences (ca. 4%). Larger (faster growing) mussels were at the more exposed sites.

The extent of shell etching (erosion) in relation to environmental variation was also examined (Hinch and Green 1988). A microcomputer-based digitizer system was used to quantify the patterns of etched periostracum and nacre. Shells of mussels from high exposure sites were proportionally more etched than were shells of mussels from low exposure sites in the same lakes (Table 1), suggesting that etching is a physical process related to water turbulence. Etching was not related to among-lake variation in water chemistry.

In 1986, the original 30 lakes were resampled, plus an additional 30 lakes which had also been surveyed by OMOE (A.P.I.O.S. 1981-83). Mussels were found in 40 of these lakes. The same relationship between shell morphology and water turbulence, as reported by Hinch and Bailey (1988), was observed. However, the variability in water chemistry among these 40 lakes was great enough (alkalinity from 22 to 1,390 $\mu\text{eq/L}$) to also affect shell morphology. Longer mussels were found in more buffered waters (Hinch *et al.* in press).

In 1986 Green began studies in Baptiste Lake on the relationship between *E. complanata* shell growth rate and form, on the one hand, and physical/chemical environment and infaunal productivity, on the other hand. Baptiste Lake is a large lake (22.3 km²) of the Algonquin Park region, in contrast to the small (average area 1.5 km²) Muskoka-Haliburton lakes studied by Hinch.

TABLE 2. Mean sizes of shells of *Elliptio complanata* from exposed and protected sides of Chocolate Drop Island, Baptiste Lake. Standard errors are enclosed in parentheses.

Side	Length (mm)	Width (mm)	Weight (g)	Age (yr)
Exposed	69.2 (1.1)	17.5 (0.4)	12.2 (0.7)	6.2 (0.3)
Protected	66.2 (1.0)	16.4 (0.3)	10.0 (0.5)	6.1 (0.2)

The preliminary research in 1986 involved SCUBA sampling around a small (~ 0.2 ha) island which is exposed to approximately 4 km of open lake on its windward shore but is protected by nearby land on its leeward shore. The shells of all mussels were measured, weighed, and sectioned for age determination. Samples for sediment and infauna were taken at the location where each mussel was collected and depth was recorded. The results indicated that shell size and shape are influenced by water turbulence, with the larger mussels on the more exposed shore (Table 2). The larger size is caused by faster growth, not by a difference in age distribution.

In summary, we have evidence of substantial intra-specific phenotypic plasticity in shell growth rate and form when mussels are subject to different natural environmental conditions. Exposure to water turbulence appears to be a particularly important factor, so that any study conducted for the purpose of assessing mussel response (e.g., growth rate or shell form) to pollution must carefully control for exposure to water turbulence.

DISTINGUISHING RESPONSE TO POLLUTION FROM RESPONSE TO NATURAL ENVIRONMENT VARIATION

The above discussion suggests that it may be difficult to distinguish effects on the shell caused by natural environment variation from pollution-related effects, at least regarding responses such as growth rate and shell form. Even in small lakes the difference between lee and windward shorelines appears to be much more important than do among-lake differences in pH covering the range 6.0-7.5 (a three-fold range in hydrogen ion concentration) and alkalinity covering the range 22-243 $\mu\text{eq/L}$ (Hinch and Bailey 1988). Results from a 1-year, three-lake reciprocal transplant experiment has shed additional light on the feasibility of using *E. complanata* as a monitor of acidification and heavy metal concentration when expo-

sure to water turbulence is held constant. Growth in the first post-transplant year was not related to the acidity of the lake to which the mussels were transplanted, but was strongly related to the source lake (in which the mussel had spent its previous life). This suggests a strong "source" effect. Soft tissue metal burdens after the first post-transplant year were also most strongly related to the source lake, as opposed to the destination lake (Hinch and Green, 1989).

An earlier study on *L. radiata* (Green 1972) found that different aspects of shell morphology responded to water turbulence as opposed to water chemistry. Such a result suggests that response to a natural environmental factor such as water turbulence may be distinguished from a pollution-related effect on water chemistry (e.g., pH and alkalinity). A similar result was found in these studies; a 40-lake comparison of shell form (Hinch *et al.* 1989) found that longer, thicker shelled mussels were related to increasing alkalinity, conductivity, and pH. Also, and with almost as strong a relationship, larger, more streamlined mussels were related to more turbulent environments.

Growth rate and shell form may be so sensitive to the environment that they are poor responses for distinguishing between natural environmental variation and change caused by pollution. Responses that are more directly linked to specific types of pollution could be employed (e.g., metal burden in tissue), but studies (refs. cited in Hinch and Stephenson 1987) have shown that, all else being equal (e.g., ambient environment metal concentrations and pH), the bioconcentration of metals by mussels is influenced by their growth rate. Thus, if mussels grow faster where there is more water movement it follows that metal concentrations in shell or tissue may differ even when concentrations in the ambient environment are the same.

There are technical problems associated with chemical analysis of the shell (discussed below). Concentration in soft tissue is the viable option at present, but that may provide a transient rather than a stable picture of the pollution situation. As Hinch and Stephenson (1987) and Smith *et al.* (1975) found, the concentration of metals in tissues does usually have some relationship to ambient metal concentration and to pH and alkalinity, but the relationship is not a simple one. It depends on the particular tissue, the particular metal and other factors (discussed below).

VALIDITY OF AGING BY USE OF SHELL GROWTH RINGS

Bivalve molluscs are especially useful for pollution monitoring because they can often be aged from annual rings in the shell. Thus, their history of exposure to the environment can be evaluated and their growth rates estimated. We have confidence in our ability to age *L. radiata* and *E. complanata*, at least for lentic populations occurring at the latitudes of north shore Lake Erie and south-central Ontario. Annual rings are usually clearer in freshwater than in marine species because there is no shell structure induced by tidal cycles. The rings are also clearer in lentic than in lotic populations of freshwater mussels because there are no rings generated by variable flow rates. We estimated ages for specimens of three species of unionids from Atlantic Canada (Metcalf *et al.* 1987). The three unionid species, as well as different sites of collection, different ring "readers," and (for one species) the results of estimates from external versus internal rings were compared. Agreement between age estimates obtained from examination of external shell bands and those obtained from examinations of thin sections was not good. The disagreement increased for older ages, as did disagreement between the two "readers."

We have used three different approaches to age unionids. The Walford Plot technique was described in Green *et al.* (1983) and in McCuaig and Green (1983). Using this technique, the lengths of the mussel at two successive external annual rings are plotted against each other, and the size-vs.-age growth curve is derived (for a sample of mussels). Thin sections of shells of some or all of the mussels are also prepared and examined to verify that the measured external rings are really annual rings. The advantage of this approach is that absolute ages of two rings need not be known, only that they are 2 consecutive years. The disadvantages are that external shell rings must be readable to some extent, and that one does not directly obtain an age estimate for an individual mussel (although one can do so indirectly by back-calculation from length).

The second approach to aging mussels is to prepare thin sections of the shells of all individuals to be aged, and count the number of internal bands (preferably several independent counts by different people). Direct counts of annual rings were done for the three unionid species from Atlantic Canada, as mentioned above, as well as *L. radiata* in

the Long Point Bay study and *E. complanata* in the Muskoka-Haliburton lakes study and in the Baptiste Lake preliminary research. This approach produces an age estimate for each mussel, but is sensitive to missing or false rings.

A third approach is to collect mussels, measure and mark them, and return them to the habitat for at least a full growing season—preferably for a full year. Then they are collected again and remeasured, yielding size-specific growth rates from which the size-vs.-age growth curve can be calculated. If the mussels are returned to the habitat for exactly a year then the Walford Plot method can be used to derive the curve. This method is the most accurate—for that given year. But that year may be an unusual one, and the mussel may be disturbed by the collection, handling, and return to the habitat. Also no direct age estimate is obtained for each individual clam.

In summary, all three methods of aging freshwater mussels have their strengths and weaknesses. A researcher should use all three methods, or however many are possible. Good agreement among age estimates, or estimated growth curves, obtained by different methods is the most convincing evidence of accurate data.

CHEMICAL COMPOSITION OF SHELL AS AN INDICATOR OF POLLUTION

A number of workers have recently suggested that analysis of contaminant concentrations in shell holds great promise and may offer several advantages over the use of soft tissues (Imlay 1982, Koide *et al.* 1982, Fang and Shen 1984, Carell *et al.* 1987). Shells may retain a record of contaminant levels over many years, and the concentration factor (shell concentration relative to ambient environment concentration) may be greater than for soft tissue. Correlations with pollution may in some cases be higher (e.g., Koide *et al.* 1982). Although the approach is promising, there are analysis problems and problems in interpretation of results (Lutz 1981, Dermott and Lum 1986).

The problems can be divided into three categories. First, there are direct analytical problems such as low contaminant concentrations and calcium interference. Second, there are problems in interpretation of results because of the influence of factors other than the ambient environmental concentration of the contaminant(s) of interest. The age and size of the mussel, its growth rate at the time of the impinging contamination, change of the

concentration of the contaminant in the shell after initial deposition (due to diagenesis for example), and metal-specific behavior in deposition can all affect the result obtained. For example, Dermott and Lum (1986) found in a study on *Elliptio complanata* that different metals behaved differently in deposition near a pollution source, in chronological trends within the shell prismatic layer, in relationship with mussel age, and in relationship with times of fastest growth rate. Third, there are problems directly related to the attempted use of chronological layers laid down in shell for the purpose of reconstruction of an environmental contamination history (over the life span of the bivalve). This is a particularly attractive possibility for unionid mussels, because most species live for at least a decade, and some live for two decades or longer. Separation of the shell into annual layers reduces the amount of material available for analysis. Furthermore, techniques which do not require separation of the shell into annual layers before analysis (e.g., probes) are less sensitive or less reliable, whereas the methods commonly used to separate annual layers can easily change the contaminant concentrations. These problems are discussed in Green *et al.* (1985).

We analyzed shells from several sources, including unionids from Lake St. Clair (courtesy of P. Hebert and C. Pugsley, U. of Windsor) and from nine waste contaminated Nova Scotia lakes (Metcalf *et al.* 1987). In both cases concentrations of metals in sediment and in mussel soft tissues were high. Metals in the shells of mussels from such obviously polluted environments should be detectable, or there would be no point in trying to apply the technique as a monitoring tool in slightly polluted environments.

Results from the Nova Scotia material were typical of the other samples. Shells of *Elliptio complanata* and *Anodonta implicata* were analyzed for As, Hg, and Pb, which were in high concentrations in soft tissue and were known pollutants in the habitats concerned. The electron microprobe (EM) and the more sensitive secondary ion mass spectrometry (SIMS) were used as "step scan" probe techniques which could estimate elemental concentrations in different shell layers across the edge of a polished thin section of shell. The EM has a sensitivity of about 200 ppm whereas SIMS can achieve a detection limit as low as 1 ppb for some elements. No reliable estimates were obtained with the EM because of its poor sensitivity. With SIMS it was possible to detect these elements using extended

counting periods, but variability was high, and the method is time-consuming and very expensive.

We also tried atomic absorption mass spectroscopy, but calcium interference was a major problem. Digestion to remove calcium but not change metal levels was difficult. Attempts were made to separate shell layers before atomic absorption mass spectroscopy analysis, using heat shock and also using oxidants. Apparently this drives off metal-containing material (perhaps organic constituents are oxidized), because the sums from the layers did not add up to the total for unseparated shell layers. Little is known about how contaminants are bound within the calcium carbonate matrix (Koide *et al.* 1982, Fang and Shen 1984, Swann *et al.* 1984).

In summary, these methodologies need greater refinement before analysis of contaminants in the shell would be a useful tool for pollution studies. Bourgoin and Evans (Trent U., personal communication) and Risk (McMaster U., personal communication) are currently developing promising techniques which include separation of the shell into nacreous and calcite layers prior to analysis. Carell *et al.* (1987) described impressive results obtained using a new analytical technique, the proton microprobe, which combines the step-scan capabilities of the EM and SIMS with high resolution and reliability.

GENETIC BASIS FOR PHENOTYPIC RESPONSE TO ENVIRONMENT

To what extent is there a genetic basis underlying the phenotypic plasticity of response of unionid mussels to varying environmental conditions? Does the genotype, as well as the phenotype, track environmental change? Morphometric and annual ring analysis of *L. radiata* from Inner Long Point Bay showed that individuals from more exposed, sandier areas were faster growing and had thicker shells (Hinch *et al.* 1986, Bailey and Green 1988). Could this spatial phenotypic variation have a genetic basis? Electrophoretic analyses indicated little divergence in frequencies of allozymes at the PGM and PGI loci over the area of Inner Long Point Bay. Several other enzymes were screened but proved to be monomorphic (MPH), not detectable (AO, LDH, G6PDH), or poorly resolved (LAP, HEX). At a larger geographic scale, there were no differences in genotype frequencies between Long Point Bay *L. radiata* and ones from Lake St. Clair and the Detroit River (data of P.

TABLE 3. Allelic frequencies at the PGM locus in three lakes.

Lake	pH	No. mussels	No. mussels with the allele			
			PGM-1	PGM-2	PGM-3	PGM-4
Tock	6.0	180	48	169	2	12
Bark	6.3	106	6	99	4	3
Beech	7.2	116	1	95	12	75
			X ² (6 df) = 161.4 **			

Hebert, Univ. of Windsor). A more distant population in Balsam Lake on the Trent-Severn waterway showed genotypic differences from the population within Inner Long Point Bay. Reproductive isolation within areas of the Inner Bay seems highly unlikely.

In two transplant experiments, one on *L. radiata* between sand and mud in Inner Long Point Bay (Hinch *et al.* 1986), and one on *E. complanata* among three lakes of differing water chemistry in the Muskoka-Haliburton area (Hinch 1987), a "source effect" was important in predicting growth rate in transplants. That is, how a mussel grew was in part predictable by where it was transplanted from. This suggests some genetic influence, but it may also just be acclimation over several years to a particular habitat which cannot be reversed in a 1-year period. Thus the juvenile mussel may grow in a manner typical of a certain environment, and continue to do so even when transplanted later in life to a different environment.

Bailey (1987) investigated the heritability of size and shape in glochidia (parasitic larvae) of *L. radiata* by comparing glochidial morphological variation among and within families (50 glochidia from each of 15 female *L. radiata*). Heritability was estimated to be 10–19 percent, which is similar to the heritability of litter size in mice and egg production in poultry, but much lower than for plant height in corn or stature in humans (65–70 percent). Heritability of larval size in marine bivalves can exceed 50 percent (reviewed in Newkirk 1980).

However, we have evidence (Hinch and Green 1989) that the populations of *E. complanata* used in the three-lake reciprocal transplant experiment differ genetically. Among-lake differences in allelic frequencies at the PGM and LAP loci do exist. Table 3 summarizes allelic frequencies at the PGM locus for the three lakes. Thus the "source effect" could have an underlying genetic basis.

Allelic frequencies deviate from a Hardy-Weinberg equilibrium in the two acidic lakes (Tock and Bark) whereas in the circumneutral lake (Beech) they do not. Acidification and associated bioavailability of metals could be responsible.

In summary, variation in morphology and growth rate in panmictic populations of *L. radiata* along the Lake Erie north shore and Lake St. Clair appears to be largely phenotypic plasticity, unrelated to adaptation in the sense of any genotypic basis. This may also be true of the morphology and growth rate variation of *E. complanata* within Muskoka/Haliburton lakes (Hinch and Bailey 1988, Hinch and Green 1989). However, there are genetic differences among lakes differing in acidification stress. Genetic selection may be occurring. Nevo and co-workers (Nevo *et al.* 1984, Baker *et al.* 1985) have published evidence of natural selection for increased resistance to mercury pollution in a marine mollusc. Allozymes at the PGM and PGI loci were involved. We have found these loci to be polymorphic in *L. radiata*, and PGM was polymorphic in *E. complanata*.

IMPACT OF KNOWN POLLUTION ON UNIONID MUSSELS

Studies of the influence of known pollution situations on growth rate, shell morphology, and soft tissue chemistry of *E. complanata* have been carried out by Hinch (1987) in Muskoka-Haliburton lakes which vary widely in their alkalinity and their vulnerability to acidification. As previously noted, one must carefully control for variation in exposure to water turbulence or that factor will outweigh the influence of water chemistry on growth rate. Hinch *et al.* (1989) found this to be true in their study of *E. complanata* shell form in 40 lakes, as did Green in recent studies on *E. complanata* in Baptiste Lake.

In a comparative study contrasting the mussels in Tock (alkalinity 22, pH 6.0) and Beech (alkalinity 238, pH 7.2) lakes, Hinch and Stephenson (1987) found that mussels from the acid-sensitive lake (Tock) had higher concentrations of Cu and Cd and lower concentrations of Zn and Mn than mussels from the circumneutral lake (Beech). These results are consistent with the theory of metals competing for binding substrates in tissue (Tessier *et al.* 1984). Beyond this generalization, the concentrations of metals were a complex function of tissue type, of the particular metal, and of the size and the age of the mussel (Smith *et al.*

1975, Green and Hinch 1986). Concentrations in gill were often twice those in the body. Both size and age of mussel generally contributed to metal concentration, but the relationship varied with tissue type and metal (independent of water chemistry).

A large-scale reciprocal transplant experiment was done among Beech, Bark, and Tock lakes (Bark: alkalinity 153, pH 6.3) in fall 1985, with controls (mussels handled, marked, and measured as were transplanted mussels, but returned to the same site in the source lake). Degree of exposure to turbulence was held constant. A year after the transplants, the mussels (including the controls) were recovered from those sites, with an 85 percent recovery rate. Growth over the year was determined, as were concentrations of selected metals. The results (Hinch 1987, Hinch and Green 1989) suggested that the situation to which the mussels were introduced (the three different lakes covering a 6.0-7.2 range of pH and a 22-238 range of alkalinity) had little effect on either the first post-transplant year of growth or on the soft tissue metal burdens as measured at the end of the first post-transplant year. Since metal analysis is destructive, there is no information about pre-transplant metal levels for these mussels; however, we have metal levels for the control mussels. Any "destination lake" effect, or "source-by-destination interaction" effects, were small relative to the "source lake" effect. Therefore, when transplant mussels are used to monitor for pollution, subsequent growth and metal burdens may be influenced by the previous history of environmental influences on the mussels and by genetic differences among the "source" populations.

Bailey and Green (1989) report density and age-structure changes over the period 1972-1986 in a population of the unionid *Anodonta grandis*, which may have been caused by hydrocarbon pollution. Overall abundance had decreased by 72 percent, and the depth distribution had shifted toward deeper water. This is based on re-sampling by Bailey in 1986 of a population that Green (1980) studied in 1972.

FUTURE RESEARCH DIRECTIONS

We are planning future research along two lines. First, we postulate that a sensitive indicator of environmental quality is unionid growth rate, which is easily measured from shell annual rings. We will model nearshore benthic productivity of

fish and infaunal benthos using unionid growth rate parameters as predictors. A pilot study has been done (Baptiste Lake) and a long-term study area has been chosen (Bay of Quinte). This approach in effect abandons the search for the "silver bullet" of a biological response which indicates a particular kind of pollution, and instead focuses on monitoring for overall environmental quality as indicated by high secondary production. Second, we will seek the "silver bullet" at the genetic level. We will try to detect locus-specific allelic response to point-source heavy metal pollution, following up on the preliminary results of Nevo and co-workers. Allelic frequencies in unionid mussels above and below discharges will be contrasted, and the dependence of lethal and sublethal responses on genotype will be evaluated in controlled experiments.

SUMMARY

1. Natural variation in growth rate, shell morphology, contaminant levels in shell and soft tissue, and other phenotypic characteristics of freshwater mussels can easily mask responses to pollution. The natural variation may be among individuals at the same location due to differences in age, size, or microhabitat, or among habitats in the same lake (e.g., differing in exposure to turbulence), or among lakes for reasons unrelated to the pollution gradient. Unless the design of studies and the statistical analyses are carefully planned, the results obtained may be uninterpretable or—even worse—misleading. Designs must be balanced with respect to mussel ages and sizes, and with respect to exposure to turbulence and the resulting substrate type differences. The only safeguard against "odd lakes" influencing results is to include enough lakes in the study to use "among lakes within a pollution level" as the error term in any tests of pollution effect. This would avoid pseudoreplication (*sensu* Hurlbert 1984) and ensure that an odd lake would have a conservative effect (reduce the likelihood of detecting a significant pollution effect) rather than a liberal one (falsely indicating a significant pollution effect).

2. The way a mussel responds to a transplant into a new environment appears to be strongly influenced by its previous environment. Therefore, any attempt to monitor an environment using introduced mussels should use mussels of the same age and size from similar habitats in the same lake as a source population.

3. Unionid bivalves can be aged reliably from shell annual rings but older individuals (> ca. 10 yr) are more difficult to age with accuracy. Estimates of age or size vs. age curves should never be based on shell external rings alone. At least some shells should be sectioned, and internal bands examined, to validate the external "annual" rings. If possible, age estimates from external rings and/or counts of internal bands should be validated by a mark-recapture experiment.

4. Techniques enabling the use of mollusc shell as record of contamination history require further development. Even if promising new techniques solve the current analytical problems, interpretation of shell contaminant concentrations must still take into account variation due to growth rate, time since deposition, and contaminant-specific deposition processes.

5. In panmictic populations, allelic frequencies do not appear to vary in any important way in response to environmental variation. Therefore variation in shell size and form within populations and among populations within the same lake is probably based on a phenotypic plasticity not determined by a varying genotype.

6. Metal burdens in soft tissue may be indicative of the concentrations of those metals in the lake, but there are complex relationships with tissue type and metal as well as mussel age and size. In addition, if the mussel is a transplant, then its metal burden will probably reflect the source lake more than the destination lake for an uncertain time period (at least a year).

7. Predictive models for environmental monitoring *can* be usefully based on responses by unionid mussels. Two approaches are suggested. General quality of environment, integrating all influences whether natural or pollution-related, can be predicted from age-specific growth rate and shell morphology. Pollution effects attributable to a particular type of pollution may be predictable using locus-specific allelic responses at the genetic level. Research to develop these two approaches is planned.

ACKNOWLEDGMENTS

Financial support was provided by the Natural Sciences and Engineering Research Council of Canada, the Ontario Ministry of the Environment, the National Water Research Institute of Environment Canada, and the Environmental Protection Service of Environment Canada. For advice, assistance in

the field and in the laboratory, and use of equipment, we thank John Forth and Bob Barnett of the University of Western Ontario Department of Geology, Paul Hebert of the University of Windsor Department of Biology, and Ron Hall and Peter Kauss of the Ontario Ministry of the Environment. Mussels and genetic data from Lake St. Clair were provided by Paul Hebert and Chris Pugsley of the Biology Department of the University of Windsor. We especially wish to thank Cynthia Walker, who worked as a technician on this research project until February 1985. Laura Kelly and Linda Stephenson were invaluable field assistants during summer 1986.

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